REMARKS

Claims 1-3, 5-10,12-15 and 29 are pending in this application. Claims 4 and 11 have been canceled without prejudice or disclaimer and claims 1-3, 5-9 and 12-15 have been amended herein. Claim 29 has been newly added.

In the amendments to claims 1-3, 5-9 and 12-15, the term "target cells" has been amended to –epithelial cells–. The term "killing" has been amended to --treating by a treatment selected from freezing, drying and irradiating– to address the rejection under 35 U.S.C. 112, second paragraph.

Support for new claim 29 may be found in the specification on pages 12-13. In particular, page 12, in lines12-14, and page 13, in lines 17, indicate that the removal of the fibroblasts prevents contamination of the epidermal cell sheet, epidermal suspension or hepatic cells that are cultured in the culture vessel. This requires removal of the fibroblasts before culturing the epithelial cells.

Regarding telephone interview.

A telephone interview was conducted between Daniel Geselowitz and Examiner Katcheves on January 28, 2003. In the interview, the rejections under 35 U.S.C. 112, second paragraph, and 35 U.S.C. 112, first paragraph, were discussed. No agreement was reached.

Claims 1-5, 7-12, 14 and 15 are rejected under 35 U.S.C. 102(b) as being anticipated by Boyce et al. (U.S. Patent No. 4,940,666).

The rejection of pending claims 1-3, 5, 7-10, 12, 14 and 15 is respectfully traversed.

Boyce et al. discloses methods and materials for generating *in vitro* populations of human

epidermal keratinocyte cells. In the Background section, it is noted that "clonal growth of human epidermal keratinocytes was obtained by plating human skin cells with a semi-confluent feeder layer of lethally irradiated 3T3 fibroblast cells ..." (column 2, lines 25-42) (Rheinwald et al. *Cell* (1975)). A later study also involving keratinocytes grown in lethally irradiated 3T3 is also noted (Rheinwald, et al. *Nature* (1977)). Other modifications of Rheinwald's systems are also discussed in columns 3-5.

Boyce discloses a basal nutrient medium MCDB153 for growing human keratinocytes. This medium is prepared from commercial chemicals as disclosed in columns 8-9. Boyce describes culturing cells in this medium in Examples 2-5 in columns 10-13.

In column 13, line 30, the reference discloses a culture prepared using Rheinwald's feeder layer method, without detailing the method. In column 13, line 66, Boyce discloses use of an irradiated 3T3 feeder layer.

Applicants respectfully submit that Boyce's MCDB153 basal medium itself does not anticipate the present claims. Applicants note that the disclosure in Boyce that is cited by the Examiner (column 2, lines 43-47 and 55) is mainly the description of the method of Rheinwald et al., which is not given in great detail in Boyce et al. Therefore, the cited disclosure of Boyce in itself does not anticipate the present claims.

In addition, Applicants respectfully submit that Boyce does **not** disclose a step of irradiating the fibroblasts in a culture vessel and then culturing the keratinocytes in the **same** ("said") culture vessel, as would be required to anticipate claim 1 and the rest of the present claims.

With regard to the Examiner's comment that an extracellular matrix would be inherent in Boyce, Applicants respectfully note that this comment in itself does not address the limitations of

claim 1. Claim 1 requires that the extracellular matrix be present **before** the target cells are cultured, and, as noted, Boyce does not even disclose that the 3T3 cells are plated before the keratinocytes.

The Examiner refers specifically to column 2, line 55, which states: "Following the establishment of primary human keratinocyte cultures by the Rheinwald et al., 3T3 feeder layer method described above, the 3T3 cells were removed on day 3 of culture with ethylene diamine tetraacetic acid (EDTA)." The Examiner apparently implies that this meets the limitation of separating 50% or more of the "treated" (as amended herein) fibroblasts of claim 2, or separating entirely in claim 3. Applicants note that Boyce et al. only discloses removal of 3T3 cells **after** they have been grown as a feeder layer for the keratinocytes. Again, this cannot anticipate the recitation of independent claim 1 or of claim 2 or 3. In addition, new claim 29 specifically recites that the separation of the treated fibroblasts occurs before the inoculation of the epithelial cells.

Applicants respectfully submit that pending claims 1-3, 5, 7-10, 12, 14 and 15 are not anticipated by Boyce et al. '666.

Claims 1-5, 7-12, 14 and 15 are rejected under 35 U.S.C. 102(b) as being anticipated by Rheinwald et al. (*Nature* Vol. 265, 1977).

The rejection of pending claims 1-3, 5, 7-10, 12, 14 and 15 is respectfully traversed.

Applicants note that Rheinwald et al. (1977) cites two previous papers by Rheinwald et al. (*Cell* 6, 317-339 (1975) and *Cell* 6, 331-334 (1975)), disclosing that when human diploid epidermal keratinocytes (of foreskin) are inoculated together with lethally irradiated 3T3 cells, the keratinocytes grow from single cells into macroscopic colonies. Rheinwald et al. (1977) discloses that mouse epidermal growth factor improves the culture lifetime of the epidermal cells.

Culture dishes were inoculated with epidermal keratinocytes and one-third the confluent density of lethally irradiated 3T3 cells (page 421, column 2). EGF was added at the first medium change, 3-5 days later.

Applicants respectfully submit that Rheinwald (1977) does not anticipate the present claims. Rheinwald states "Culture dishes (50 mm) were inoculated with $5x10^3$ epidermal keratinocytes **and** one-third the confluent density of lethally irradiated 3T3 cells" (emphasis added). Applicants note that the 3T3 cells are specifically inoculated at "one-third of the confluent density," implying that they are being transferred from another vessel. There is no disclosure of removal of any 3T3 cells at the time of inoculation.

Therefore, Rheinwald (1977) does **not** disclose culturing the keratinocytes in the **same culture vessel** as the 3T3 cells were irradiated in, as is required by claim 1.

Moreover, Rheinwald (1977) does not disclose any separation of the 3T3 cells before adding the keratinocytes. In Rheinwald (1977), the 3T3 cells are separated three days after the inoculation with the keratinocytes. New claim 29 specifically recites that the separation of the treated fibroblasts occurs **before** the inoculation of the epithelial cells.

Applicants respectfully submit that pending claims 1-3, 5, 7-10, 12, 14 and 15 are not anticipated by Rheinwald et al. (1977).

Claims 1-12, 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boyce et al. (U.S. Patent No. 4,940,666).

The rejection of pending claims 1-3, 5-10, 12, 14 and 15 is respectfully traversed.

Applicants have noted above that the relevant disclosure in Boyce appears to be the

discussion of the Rheinwald method in column 2 and the examples in columns 13-14. The only actual experimental detail appears to be in column 13, line 66, to column 14, line 2. However, this portion of the reference only states that a 60 mm petri dish "received inocula of 100 cells/cm^2 prepared from a stratified primary culture initiated with an irradiated 3T3 feeder layer ..." No procedure appears to be given for preparing the irradiated 3T3 feeder layer. Therefore, the teaching of Boyce cannot be considered to be different from that of the Rheinwald (1977) article cited in Boyce. Applicants therefore assert that there is no suggestion in Boyce et al. for the recitation of independent claim 1 or of the dependent claims.

Applicants also submit that there is no suggestion in Rheinwald (1977) (cited in Boyce and discussed above) for the specific limitation of claim 1 that requiring the step of treating the fibroblasts in a culture vessel and **then** culturing the keratinocytes in the **same** ("said") culture vessel. Rather, Rheinwald (1977) discloses inoculating the keratinocytes "**and**" the lethally irradiated 3T3 cells, implying that these are being inoculated **at the same time**. Since the lethally irradiated 3T3 cells are **inoculated** at this time, it is apparent that the lethally irradiated 3T3 cells and the keratinocytes are not cultured in the same vessel as the 3T3 cells were irradiated in. There is no suggestion or motivation in the reference for this limitation of claim 1.

Applicants again respectfully submit that the Examiner's comments about the extracellular matrix being inherent are irrelevant because they do not address the limitations of claim 1, which requires the extracellular matrix on a surface of the culture vessel, "**and then**" culturing the epithelial cells in the culture vessel.

With regard to the limitations of claims 6 and 7 requiring repeating the treating of the fibroblasts, the Examiner cites no suggestion in the cited references, but states (page 5 of the Office

action) that "One of ordinary skill in the art would be motivated to repeat said step in order to confirm that the fibroblast cells are killed ..." Applicants respectfully disagree. Lethal irradiation is conventionally performed with delivery of specific doses determined, typically, by setting equipment parameters. Applicants are not even certain how one might irradiate cells, test how "killed" they were, and then irradiate them some more until they were sufficiently "killed"; certainly this is not conventionally done in the art.

In addition, claim 8 requires two treatments, one of which would be other than irradiation. The Examiner has provided no suggestion in the prior art for this limitation, and Applicants respectfully submit that no suggestion for this limitation can be found in Boyce '666 or Rheinwald (1977).

Applicants therefore assert that pending claims 1-3, 5-10, 12, 14 and 15 are novel and non-obvious over Boyce et al. '666.

Claims 1-15 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention.

The rejection is overcome in part by the amendments to the claims, and is traversed in part.

The Examiner indicates that the term "killing" renders the claims indefinite. The term "killing" in the claims has been amended to treating by a treatment selected from freezing, drying and irradiating. This recitation is supported by claim 4, which has been canceled.

The Examiner states that the term "derived from a mammal" is indefinite because "derived" is non-specific. Applicants respectfully argue that the phrase "derived from a mammal" would be

well understood by one of skill in the art, and reconsideration of this rejection is respectfully requested. In the present state of the art, all living cells are obtained in a lineage from living cells, and in the case of mammalian cell lines, this lineage extends back to cells obtained from a mammal.

The Examiner refers to "derivations" to produce these cells. The only required "derivation" is the obtaining of the original cells of the lineage from the mammal. Any other treatments to cells during the growth of the lineage are irrelevant to the definition. Applicants therefore assert that the limitation "derived from a mammal" is well defined.

Claims 1-15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for epidermal target cells, does not reasonably provide enablement for hepatic target cells, as in claim 13.

The rejection of claims 1-15 under 35 U.S.C. 112, first paragraph, is respectfully traversed.

Applicants respectfully note that the claims have been amended herein to replace the term "target cells" with –epithelial cells–, for clarity. Applicants respectfully submit that claims 1-12, 14 and 15, both before and after the present amendment, did not specifically recite "hepatic" target cells, and that these claims both before and after the present amendment were fully enabled by the specification for what they recited.

Claim 13 does specifically recite hepatic cells. Applicants respectfully submit that all that is necessary in order to perform the claimed invention with the target cells being hepatic cells is to culture hepatic cells in the culture vessel of claim 1. The only change that this would require relative to the specific examples of the specification (pages 15-19) would be the addition of hepatic cells rather than epidermal cells to the culture vessel. This may also be inferred from the specification on

page 12, lines 14-15, which implies that the procedures using epidermal cell sheets, epidermal cell suspensions or hepatic cells are similar. Epidermal cell sheets and suspensions are also discussed in more detail on pages 14-15. One of skill in the art would certainly be able to obtain hepatic cells and inoculate hepatic cells in a similar manner to epidermal cells. Claim 13 is therefore fully enabled.

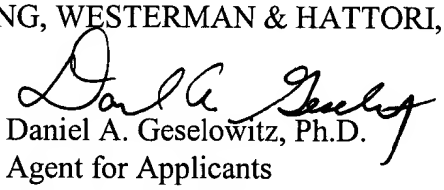
If, for any reason, it is felt that this application is not now in condition for allowance, the Examiner is requested to contact Applicants undersigned agent at the telephone number indicated below to arrange for an interview to expedite the disposition of this case.

Attached hereto is a marked-up version of the changes made by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

In the event that this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. Please charge any fees for such an extension of time and any other fees which may be due with respect to this paper, to Deposit Account No. 01-2340.

Respectfully submitted,

ARMSTRONG, WESTERMAN & HATTORI, LLP



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Please cancel claims 4 and 11 without prejudice or disclaimer and amend claims 1-3, 5-9 and 11-15, as follows:

1. (Thrice Amended) A method for adhering and proliferating ~~target~~ epithelial cells, which comprises the steps of:

inoculating, culturing and then ~~killing~~ treating by a treatment selected from freezing, drying and irradiating, in a culture vessel, fibroblasts derived from a mammal, so as to leave an extracellular matrix on a surface of said culture vessel, and then

culturing the ~~target~~ epithelial cells in said culture vessel.

2. (Twice Amended) The method according to Claim 1, wherein 50% or more of ~~said killed~~ the treated fibroblasts are separated from the culture vessel.

3. (Twice Amended) The method according to Claim 1, wherein ~~said killed~~ the treated fibroblasts are separated from the culture vessel entirely.

5. (Twice Amended) The method according to Claim 1, wherein said fibroblasts are ~~killed~~ treated by at least one selected from the group consisting of β ray, γ ray, X-ray, electron beam and UV ray.

6. (Twice Amended) The method according to Claim 1, wherein said ~~killing~~ treating step of fibroblasts comprises repeating one treatment selected from the group consisting of freezing, drying and irradiating.

7. (Twice Amended) The method according to Claim 1, wherein said ~~killing~~ treating step of fibroblasts comprises repeating exposure to at least one selected from the group consisting of β ray, γ ray, electron beam, UV ray and X-ray.

8. (Twice Amended) The method according to Claim 1, wherein said fibroblasts are ~~killed~~ treated by a combination of at least two treatments selected from the group consisting of freezing, drying and irradiating.

9. (Twice Amended) The method according to Claim 1, wherein fibroblasts are ~~killed~~ treated by at least one selected from the group consisting of β ray, γ ray, electron beam, UV ray and X-ray.

12. (Twice Amended) The method according to Claim ~~11~~ 1, in which said epithelial cells are epidermal cells.

13. (Twice Amended) The method according to Claim 1, wherein said ~~target~~ epithelial cells are hepatic cells.

14. (Twice Amended) An epidermal cell sheet prepared from ~~target~~ epithelial cells that are epidermal cells, cultured using the method according to Claim 1.

15. (Twice Amended) An epidermal cell suspension prepared from ~~target~~ epithelial cells that are epidermal cells, cultured using the method according to Claim 1.